EFFECT OF PH AND ENZYME LOADING ON THE PROTEIN CONCENTRATION DURING COLLOCALIA FUCIPHAGA HYDROLYSIS

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ABSTRACT

The purpose of this study is to investigate the effect of pH and enzyme loading on the concentration of protein during enzymatic hydrolysis of *Collocalia Fuciphaga*. *C. Fuciphaga* is refers to the bird nest from one swiftlet species that is used as the sample known to be highly nutritious. Bovine Serum Albumin (BSA), a standard protein solution was used as the benchmark for the reference of the protein concentration. A research had been proposed in this study to optimize the production of protein from *C.Fuciphaga.* The effect of changing of the parameters values during the enzymatic hydrolysis was studied and the higher protein concentrations produced were highlighted. The standard protein curve of Bovine Serum Albumin (BSA) is prepared first with several dilutions for protein standard curve. The results from two significant parameters in the experiment was taken and analyzed. The selection of parameters values was taken from the enzymes' optimum condition where the pH value of Alcalase 2.4L is from pH 7 until pH 10 whiles the temperature is fixed at 50^{o}C. Enzyme loading was varies from 1.5% to 3.0% (v/w). The sample was analyzed using UV-Vis Spectrophotometer to determine the concentration of protein in each sample. The optimum condition is found to be at extraction condition of pH 8.5 and enzyme concentration of 3.0% (v/w). Characterization of the sample had been done and validated the protein composition on the extracted sample.

ABSTRACT

Tujuan kajian ini adalah untuk mengkaji kesan pH dan kepekatan enzim pada kepekatan protein semasa hidrolisis enzim *Collocalia Fuciphaga*. *C. Fuciphaga* adalah merujuk kepada sarang burung dari satu spesies burung walit yang digunakan sebagai sampel kerana ia dikenali untuk menjadi sangat berkhasiat. Serum Albumin Bovine (BSA), larutan protein piawai telah digunakan sebagai penanda aras untuk rujukan kepekatan protein. Satu penyelidikan telah dicadangkan dalam kajian ini untuk mengoptimumkan pengeluaran protein dari *C.Fuciphaga*. Kesan perubahan nilai parameter semasa hidrolisis enzim telah dikaji dan kepekatan protein yang lebih tinggi yang dihasilkan telah diketengahkan. lengkung protein standard Bovine Serum Albumin (BSA) disediakan terlebih dahulu dengan pencairan beberapa sebagai lengkung protein piawai. Keputusan dari dua parameter signifikan dalam eksperimen akan diambil dan dianalisis. Pemilihan nilai parameter telah diambil dari keadaan optimum enzim di mana nilai pH Alcalase 2.4L adalah dari pH 7 sehingga pH 10 manakala suhu ditetapkan pada 50^{o}C. Keadaan optimum didapati berada pada keadaan pengekstrakan pH 8.5 dan kepekatan enzim sebanyak 3.0% (v / w). Sampel ini kemudiannya akan dianalisis menggunakan Spectrophotometer UV-Vis untuk menentukan kepekatan protein dalam setiap sampel. Pencirian sampel telah dilakukan dan mengesahkan komposisi protein pada sampel yang diekstrak.

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LIST OF ABBREVIATIONS

EBN	Edible Bird Nest
FTIR	Fourier Transform Infrared Spectroscopy
OFAT	One Factor at a Time
DNS	Dinitrosalicylic acid
BSA	Bovine Serum Albumin
AU	Anson Unit
NaOH	Sodium Hyroxide
OD	Optical Density
rpm	Round per Minute
SEM	Scanning Electron Microscopy

LIST OF SYMBOLS

^{o}C	-	Degree Celsius
%	-	Percent
g	-	Gram
L	-	Liter
rpm	-	Rotation Per Minutes
M	-	Molarity
ml	-	Milliliter
v	-	Vibration wavelength
cm^{-1}	-	Reciprocal Centimeter
mg	-	Milligram
Abs	-	Absorbance
ml	-	Millilitre
min	-	Minute
hr	-	Hour

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Proteins are essential food components because they are a source of amino acids needed for growth and maintenance and provide functional properties to foods. Commercially available protein foods are obtained from a range of animal and plant sources and are used as functional ingredients (Martina. 2002). Due to the increasing costs and limited supplies of animal proteins and since vegetable protein is the most abundant source of protein on the Earth, a number of vegetable proteins such as alfalfa leaf, cottonseed, winged bean, peanut and soya have been investigated for possible incorporation into formulated foods.

Protein molecules tend to unfold and even become fully denatured under unfavoured conditions, such as a high temperature, an acidified condition, a high pressure or even excessive shear. Denatured protein molecules will aggregate and/or

crosslink to form larger clusters and, at high concentrations, will form a three-dimensional solid-like network (or a gel). Therefore, proteins are regarded as one of the main classes of building blocks used in many semi-solid foods for conferring mechanical properties (Linqiang et. al., 2008).

For this particular research, the raw material that will be used is *Collocalia Fuciphaga*. *C. fuciphaga* species is popularly known as the White Nest or House Nest swiftlet. *C. fuciphaga* measures about 12 cm in its entire length and weighs about 15 to 18 gm. In recent years, hormone-like substances such as mitogen and avian epithelial growth factor have been found in *C. fuciphaga* (Jie *et al*., 2009).

Edible bird's nest (EBN) is the nest of the swift and is constructed with salivary glue, which is a cementing substance, and may incorporate other materials such as vegetation or feathers. Although EBN mainly contains carbohydrates, amino acids, and mineral salts, the abundant ingredient is composing of glycoproteins (Wu , Wu et al., 2010). Due to its nutritious and medical properties, EBN has been deemed a precious food tonic in Chinese community ever since the Tang (907 AD) and Sung (960–1279 AD) dynasties. Despite the long history of using EBN for medicinal purpose, there have only been a limited number of scientific reports on the health benefits of EBN.

1.2 PROBLEM STATEMENT

The problem of the research is to find out the most appropriate condition that can optimize the protein yield via the enzymatic hydrolysis process. The important part is to search for the protein sources that can be produced in a great amount so that the nutrients from the food can be obtained by all people who in need. Hence the nutritional food is widespread produced all around the world to get all of the healthy life with all of those nutritional benefits. However, the protein extracted from marine life, sometimes not suitable to consumers with heart diseases and blood pressure.

With the researches that had been conducted nowadays, protein from various sources; from plant or from other animal is being looked out. Besides, the most optimum production of the nutritious food is being focused nowadays to produce an optimum condition for the extraction of the protein. The research regarding the optimum condition of the protein concentration of *Collocalia fuciphaga* has not been done before.

1.3 OBJECTIVES

The main objective of this research is to characterize and optimize protein extraction yield from *C.fuciphaga* using enzymatic hydrolysis method. In this research also have a few specific objectives. The specific objectives are:

- To determine the influence of pH on extraction protein yield.
- To determine the influence of enzyme loading on extraction protein yield.
- To characterize the sample by using Fourier Transform Infrared Spectroscopy (FTIR).

1.4 SCOPES OF THE STUDY

Below are the scopes of the study for the research that will be conducted;

- This research will be focusing on the characterization of *C.fuciphaga*mainly using the Ultra-violet Visible spectrophotometer.
- Study the effect of three parameters, the pH of the solution from pH 7-10, and the enzyme loading ranging from 1.5% to 3.0
- The optimal condition of pH, and enzyme loading during the enzymatic hydrolysis will be determined by using Lowry Method One Factor at a Time (OFAT).

1.5 SIGNIFICANCE OF THE PROPOSED STUDY

Nowadays the application of protease is often an attractive means for obtaining better functional properties of food proteins, without impairing their nutritional value. In order to increase and to improve the protein quality produced from the sources of protein, the extracted protein need to undergo the hydrolysis process with the addition of enzyme with several modifications made (Ng et al., 2012). The process of protein extraction using enzymatic hydrolysis method is proved to produce hydrolysates with well-defined peptide profiles (Palupi et al., 2010).

Hence, the study of the extraction protein from Collocalia Fuciphaga with the optimum condition will be conducted so that the nutrients on the protein in the bird nest will be preserve and produce higher protein yield. Besides people that doubtful with the contents of the nest especially the Muslim and also Vegetarian users will leave the doubt and take the benefits of the bird nest. As the sample taken is not from the marine life or any animal sources, so it does not causes allergic to the consumer.

Hence it is really suitable for all type of people who need it.

CHAPTER 2

LITERATURE REVIEW

2.1 SOURCES OF PROTEIN

Protein is essential for the development of the human life as they are really needed for the growth and maintenance of the body. They are sources of amino acids that responsible for many functional properties that influence to foods. The basic structures of protein are composed of small units. The units are consists of small units which are the amino acids units that are also called the building blocks of protein. Protein is an essential nutrient for human many living things. Protein is contained in every part of your body, the skin, muscles, hair, blood, body organs, eyes, even fingernails and bone. Protein is the most plentiful substance in your body after water and no life can survive without protein (Lauritzen, 1992). There are various sources of protein that can be taken nowadays from a two different type of protein sources.

The sources can be classified as the animal protein and the plant protein. Protein does exist for both animal and plant sources. Different type of animal and plant sources have different amount of protein in it. The determination of protein in various sources had been discovered and continuously in the research to know the protein concentration and the quality of the protein. Hence nowadays, a wide range of protein from animal and plant are being commercialized and are available to be used today to be used as useful ingredient for general and specific uses (Hoffman & Falvo, 2004). Protein is however not a primary source of energy but it can be used as energy where the protein have to be metabolized into the much more simplest form into the amino acid from in order to generate energy. (Hoffman & Falvo, 2004)

Figure 2-1 Example of different Sources of Protein

2.1.1 Plant as a Sources of Protein

The source of protein from plant is being nowadays being the alternatives for the consumption rather than the protein from animal. The protein on plants can be obtained

from a lot of sources. Whole grains and cereals are another source of protein. There are a lot of sources of protein that can be obtained nowadays. Proteins from plants contribute over 65 percent on the worldwide basis on the per capita supply of protein (Young & Pellen, 1994) Oats, maize, rice and wheat are examples of protein sources which the concentration of protein is greater than 7 percent (Young & Pellen, 1994). For the vegetarian sources of protein the sources include the legumes, nuts, seeds and fruits. Some of the vegetarian foods with higher concentration of protein include soybeans, lentils, white beans, pigeon peas, almonds, sunflower seeds, cowpeas, walnuts and pumpkin seeds.

Determination of protein in plant had been made in determining the quality and the digestibility of the protein. The plant consider as incomplete protein because of the protein is not having the all the amino acid that should be consumed in human body. According to Hoffman & Falvo (2004), the protein form plant said to be having less protein from animal protein and are generally lacking single or two amino acids. Vegetable protein is the alternative choices for those who are really want to have a result in a reduction in the intake of the saturated fat and cholesterol.

2.1.2 Animal Source of Protein

Proteins from animal sources is the highest quality food sources that been consume on today's life. The protein that are taken from protein is has the quality higher than the protein from the plant sources. The protein from animal can be consumed from

a lot of sources such as meat, egg, fish, poultry and milk. These protein sources of food have been consume by almost all of the human population around the world for the consumption of the protein in their everyday diet. Based on the statement made by (Hoffman & Falvo, 2004), the protein from animal has the highest quality rating due to the protein in the animal has all the required characteristics as the protein needed. The protein is said to be in the completeness for the animal protein. Also for the benefits taken from total protein consumption, a lot of elderly people had also been benefited from consuming animal sources of protein. Meat consisting diets had been resulted in greater gains in lean body mass compared to subjects on a lactoovovegetarian diet (Campbell et al., 1999).

The animal protein is said to be having a higher contents of protein than the protein in the vegetable plant protein. A high animal protein diets in the daily intake had been shown to give a much greater net protein synthesis than a high vegetable protein diet (Pannemans et al., 1998). This statement supports the information that protein is much better in the protein content rather than the plant protein. Hence the protein content synthesis from the plant is less compare to the content of protein in the animal.

2.1.3 Amino Acid in Animal

Amino acid commonly known to be 20 different types of amino acids those are different from each other. Each different protein is composed of various amino acids put together in varying order with almost limitless combinations. Most proteins are large molecules that may contain several hundred amino acids arranged in branches and

chains (Lauritzen, 1992). The protein sources can be divided into two major classes that are plant sources and animal sources protein. The protein in animal is a complete source of amino acid where all the amino acids that are essential to our body can be taken from the animal sources. This shows that the protein in the plant generally lack one or more amino acids in it. People who want to take benefits from the plant in term of taking the protein from it have to take several type of vegetables or plant to take all the required amino acids in their everyday diet (Hoffman & Falvo, 2004).

For the 20 amino acids that had been identified, they are all needed for human growth and metabolism. However Hoffman and Flavo (2004) stated that in the total of 20 types of amino acids, twelve of these amino acids or eleven in children are nonessential amino acid. The nonessential amino acid meaning that our body can produce the amino acid on our own body without consuming in the everyday diet. The synthesis of the amino acid can be synthesis by the human body by its own without having to be taken from other sources. For the other remaining amino acids, they cannot be synthesized in the body and are described as essential amino acid meaning that they have to be consumed in our diets. Without any of these amino acids, problems will occurs where it will compromise the ability of tissue to grow, be repaired or be maintained (Hoffman & Falvo, 2004).

2.1.4 Types of Amino Acids

There are several types of amino acid that are being the general classification of the amino acids. There are classified based on the different functional group attached to the amino acid with different location with one to another. The carboxylic acid group and amine group attached at the on the same location to the first, or alpha, carbon atom next to the –COOH group (Clark, 2011). The amino acid types can be classified based on the general statement of the requirement on dietary scope of some amino acids. However due to the different amount of the requirement of the amino acid, the classification is hard to be made.

Table 2-1 Twenty Types of Amino Acids with Classification (Volpi et al., 2013)

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Aspartate
Lysine	Asparagine
Methionine	Glutamic acid
Phenylalanine	Glutamine
Threonine	Glycine
Valine	Serine
	Tyrosine

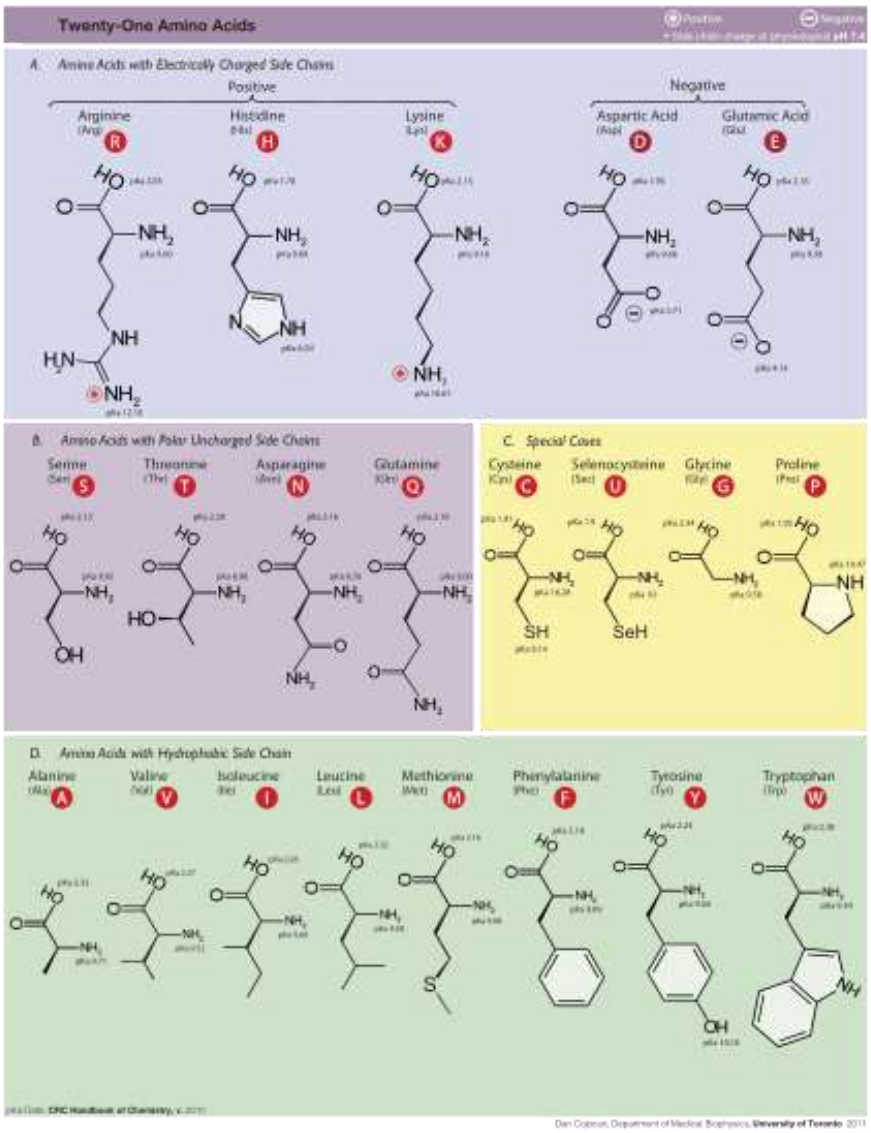

Figure 2-2 Twenty amino acid overview diagram (www.wikipedia.com)

2.2 APPLICATION OF AMINO ACID

The amino acid had been used in many industries especially the food industry. The food industries had been benefited a lot from the technology regarding the amino